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TECHNICAL GUIDE

Aerobic Biodegradability in Organic Substances:

A Top-Down Approach for Estimating Measurement Uncertainty

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Cooperating institution

Physikalisch-Technische Bundesanstalt (PTB)
German National Metrology Institute
Bundesallee 100
38116 Braunschweig, Germany
www.ptb.de

Participants

Preparation of the document:

Lic. Mariana Papa, *Researcher, Department for Handling and Management of Chemical Substances (DMyGSQ), INTI.*
e-mail: mpapa@inti.gob.ar

Lic. Soledad Barbelli, *Researcher, Department for Handling and Management of Chemical Substances (DMyGSQ), INTI.*
e-mail: barbelli@inti.gob.ar

Lic. Jimmy Venegas Padilla, *Researcher, Chemical Metrology Department, LCM.*
e-mail: jvenegas@lcm.go.cr

Organizing committee:

Franziska Kamm, *Project Coordinator, PTB.*

Beatriz Paniagua *Valverde, Consultant, PTB.*

Technical review of the guide:

Lic. Marisa Delbon, *Researcher, Department of Measurement Quality, INTI.*

Lic. Silvina Aued, *Researcher, Department of Measurement Quality, INTI.*

B. Ricardo Irigay. *Researcher, Department of Water Quality and Environmental Assessment, LATU.*

Eng. Gabriel Molina Castro, *Researcher, Chemical Metrology Department, LCM.*

PhD. Bryan Calderón Jiménez, *Researcher, Chemical Metrology Department, LCM.*

Table of Contents

1. Background

The right to a healthy environment was recognized in the *Stockholm Declaration* of 1972, which was ratified by most countries worldwide and was extended and ratified again at the *Rio Conference* in 1992, where the focus continued to be placed on the concept of sustainable development, given the evidence that human activities in pursuit of economic growth were responsible for the main environmental threats (Devia, Krom, & Nonna, 2019). It was at that conference that leaders signed the *Convention on Biological Diversity*, whose main objective is the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of benefits arising from the use of genetic resources (UN, 1992). Another important milestone was the signing of the *Escazú Agreement* which recognizes the right to information, participation and justice in environmental matters for the countries of Latin America and the Caribbean (UN, 2018).

With the aim of ratifying the *Conventions and Agreements* designed to preserve the environment, new mechanisms have been developed at the global level to mitigate the environmental impact produced by human activities. In order to handle, manufacture and sell chemicals, it is now necessary to comply with standards and regulations aimed at protecting health and the environment; for example, the European market is governed by the REACH regulation (European Chemicals Agency, 2022), which applies to all chemical substances used in industrial processes and those present in everyday products (European Parliament and Council, 2020). Once chemical agents have been produced and are marketed globally, they must be classified according to the Globally Harmonized System (GHS), which establishes criteria for pure substances and mixtures, in order to classify and label chemicals based on the hazards they represent to health and the environmental (UN, 2017).

In order to strengthen the region in the measurement of parameters of environmental relevance, the German Federal Ministry for Economic Cooperation and Development (BMZ), through the National Metrology Institute of Germany (PTB) and within the framework of the Regional Quality Infrastructure Fund for Biodiversity and Climate Protection in Latin America and the Caribbean (PTB, 2014), decided to finance the development of the regional project in Latin America entitled "Quality assurance in the measurements required to determine the biodegradability of chemicals". This project served to harmonize technical criteria and implement the "ready" aerobic biodegradability test using the biochemical oxygen demand (BOD) method of analysis, or Closed Bottle Test (ISO 10707:1994), with the respective quality assurance to ensure the validity of the results, while the measurement comparability of the biodegradability of cleaning products was evaluated through a proficiency testing (LACOMET, 2019).

This guide provides the scientific and technical community with a simple practical and economical strategy for estimating uncertainty through the "top-down" approach, which allows them to use quality control data or data from experiments to verify "ready" biodegradability tests. The knowledge acquired during the project execution, the measurements carried out by different laboratories and the development of proficiency testings provided inputs and information of the utmost importance for estimating uncertainty for the "ready" biodegradability test using biochemical oxygen demand analysis (ISO 10707:1994), or the equivalent method, the OECD 301D Closed Bottle Test (OECD, 1992).

2. General and specific objectives

2.1. General objective

Create a technical guide for estimating measurement uncertainty based on a "top-down" analysis approach that serves as a metrological input for testing laboratories when measuring the "ready" biodegradability of water-soluble organic substances.

2.2. Specific objectives

1. Establish guidelines for the correct identification and quantification of the sources of measurement uncertainty when measuring "ready" biodegradability using a "top-down" approach.

2. Estimate the measurement uncertainty in the "ready" biodegradability test for water-soluble organic substances present in cleaning products, using historical data as a source of precision uncertainty and interlaboratory comparisons as a source of bias uncertainty.

3. Abbreviations and symbols

3.1. Abbreviations

C	Carbon
COD	Chemical Oxygen Demand
CRM	Certified Reference Material
DMyGSQ	Department for Handling and Management of Chemical Substances
DOC	Dissolved Organic Carbon
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GUM	Guide to the Expression of Uncertainty in Measurement
IC	Intercomparisons; interlaboratory comparison
INTI	Argentine National Institute of Industrial Technology
ISO	International Organization for Standardization
LCM	Costa Rican Metrology Laboratory
OECD	Organization for Economic Cooperation and Development
PT	Proficiency testing
REACH	Regulation on the Registration, Evaluation, Authorisation & Restriction of Chemicals
RMS	Root Mean Square error
SS	Suspended solids
$ThCO_2$	Theoretical Carbon Dioxide
ThIC	Theoretical Inorganic Carbon
ThOD	Theoretical Oxygen Demand

3.2. Symbols

q	Number of replicates required by the measurement method
k	Coverage factor of expanded uncertainty
s	Standard deviation
u_c	Combined standard uncertainty
u_i	Standard uncertainty of the i^{th} component
U	Expanded uncertainty
n	Number of independent data or replicates per sample
m	Number of samples used to estimate the average range
p	Number of satisfactory results in a PT or IC
$\bar{x}$	Arithmetic mean
x_i	Value of the i^{th} datum
$x_{ref\ PT}$	Reference value or value assigned in a PT or IC
$x_{lab\ i}$	Result of the i^{th} participant
s_L	Intermediate precision variance associated with the factor
s_r	Variance due to repeatability
s_M	Variance due to repeatability of routine samples

NB: For a better understanding of this guide, we recommend consulting the definitions provided in the International Metrology Vocabulary (JCGM 200, 2012).

4. Introduction

In recent years the commercial dynamics of products and services have included the factor of environmental sustainability. Over the last few decades, the linear production and consumption model has gradually begun to be replaced by the circular economy model, which is based on the principles of ecological economy and industrial ecology (Wautelet, 2018). This theoretical framework focuses on the interdependence of the human economy and ecosystems, enabling the integration of social, ecological and economic goals (Gutberlet, Carenzo, Kain, & Martiniano de Azevedo, 2017).

The cleaning products industry uses a wide range of chemicals including surfactants, complexing agents, solvents and fragrances (McCabe, Clement, & Ochoa, 2008). In addition to being the main components of cleaning products, surfactants in particular are widely used due to their multiple applications in various areas, such as agriculture and the oil, textile, cosmetic and pharmaceutical industries (Schramm, Stasiuk, & Marangoni, 2003). It is important to emphasize that cleaning products can sometimes contain chemicals that may cause or exacerbate health problems (allergies, asthma, endocrine disruption) or environmental damage (Jardak, Drogui, & Daghrir, 2016). For this reason, ecosystems may be affected by the use of persistent chemicals and the discharge of untreated effiuents (Acir & Guenther, 2018; Rebello, Asok, Mundayoor, & Jisha, 2014; Danish Agency Environmental Protection, 2001). Assessing the potential environmental danger of the organic chemicals present in cleaning products using biodegradability tests has proved fundamental in demonstrating that the changes in production models have less impact on ecosystems (OECD, 2006). Both regulations on use as well as chemical labelling systems require the evaluation of biodegradability. Furthermore, these measurements are essential for researching the potential for bioaccumulation and ecotoxicity of organic chemicals in organisms belonging to different trophic levels, as they provide tools to clarify the possible effects on ecosystems in a comprehensive manner (UN, 2017).

There is a wide variety of tests used to evaluate biodegradability, which can be classified into three main groups, and which, according to the guidelines proposed in the OECD (2006), constitute sequential stages in the biodegradability analysis of an organic substance (see **Fig 1**). The first group is composed of *ready biodegradability tests*, which are the most stringent, since they use inocula (microorganisms) not adapted to the sample being evaluated and provide information on the degree of ultimate degradation in most aquatic environments, including effiuent treatment systems. The next group consists of *inherent biodegradability tests*, which offer more favourable conditions for degradation and can be carried out using adapted microorganisms. Finally, we have *simulation tests*, which incorporate different environmentally relevant conditions in order to determine the degradation rate; they tend to use native microorganisms and the results obtained are limited to the conditions tested.

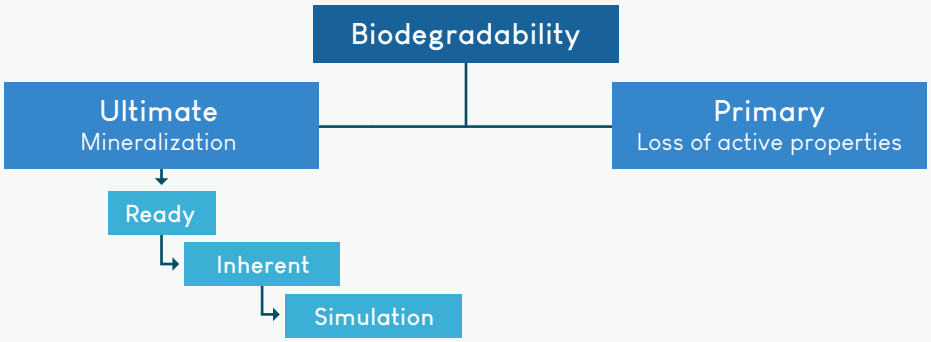

Figure 1. Classification of biodegradability tests: *ultimate biodegradability and primary biodegradability*. The arrows show the sequence of analysis suggested by OECD (2006).

The OECD guidelines describe seven methods of *ready biodegradability*, the main characteristics of which are summarized in **Table 1** (OECD, 1992; OECD, 2006). As they constitute the first step in evaluating the biodegradability of organic chemicals, these tests are widely used, while inherent biodegradability or simulation tests are considered second-tier evaluations, intended for substances that are not easily biodegradable, or for research aimed at identifying where they end up in a specific environment.

Table 1. Main characteristics of the different ready biodegradability test methods (OECD, 1992; OECD, 2006).

Method	Incubation conditions	Test volume	Concentration of the substance to be evaluated	Inoculum source	Inoculum concentration	Cut-off level
OECD 301 A ISO 7827: 1994	28 days, aerobic, agitated.	(0.25-2) L	(10-40) mg/L DOC	Activated sludge, sewage effiuent, surface water, soil or a mixture thereof.	< 30 mg/L of settled effluent	70% DOC Removal
OECD 301 B	28 days, aerobic, continuous aeration.	(2-5) L	(10-20) mg/L DOC		< 100 mL effluent/L (10^7-10^8) cells/L	60% $ThCO_2$ production
OECD 301 C	28 days, aerobic, agitated	Respiro-meter	100 mg/L	Mix of fresh samples of sewage or industrial effiuents, activated sludge, surface water or soil.	< 30 mg/L of settled effluent (10^7-10^8) cells/L	60% of ThOD
OECD 301 D ISO 10707: 1994	28 days, aerobic, static.	300 mL	(2-10) mg/L or (5-10) mg/L ThOD	Derivative of secon-dary effiuent from the treatment of effiuents or activated sludge on a laboratory scale, pre-dominantly from domes-tic effiuents. Alternatively, surface wa-ter, soil, etc.	< 5 mL effluent/L (10^4-10^6) cells/L	60% of ThOD

Method	Incubation conditions	Test volume	Concentration of the substance to be evaluated	Inoculum source	Inoculum concentration	Cut-off level
OECD 301 E ISO 7827: 1994	28 days, aerobic, agitated. Medium with trace elements and growth factors.	(0.25-2) L	(10-40) mg/L DOC	Derivative of secondary effiuent from the treatment of effiuents or activated sludge on a laboratory scale, predominantly from domestic effiuents.	< 0.5 mL effluent/L 10^5 cells/L	70% DOC Removal
OECD 301 F ISO 9408: 1999	28 days, aerobic, agitated.	Respirometer	100 mg/L ThOD or (50-100) mg/L ThOD	Activated sludge, sewage effiuent, surface water, soil or a mixture thereof.	< 30 mg/L of settled effluent < 100 mL effluent/L (10^7-10^8) cells/L	60% of ThOD
OECD 310	28 days, aerobic, agitated.	125 mL	(20-40) mg/L DOC	Activated sludge, sewage effiuent, surface water, soil or a mixture thereof.	(4-30) mg/L of SS or 10% v/v of secondary effluent	60% ThIC production

The selection of the most suitable method for evaluating biodegradability depends on the intrinsic properties of the compounds, the conditions of use and the means of disposal into the environment. These variables and the scope of each methodology are described in the standard (ISO/TR 15462:2006). In all cases, the substance to be evaluated constitutes the only exogenous carbon source, and quantification is carried out using a non-specific analytical measurement, thanks to which they can be applied to a wide variety of compounds without the need to implement specific analytical procedures (OECD, 2006). The analytical methods used also quantify the degradation of intermediate products, so the result obtained corresponds to the *ultimate biodegradability*, that is, the transformation of organic matter into CO_2, H_2O, mineral salts and biomass (see **Fig. 2**). A chemical can be classified as readily biodegradable when it achieves a 60% reduction

in theoretical oxygen demand or theoretical CO_2 production, or 70% in the case of dissolved organic carbon (DOC). These percentages correspond to a virtually complete degradation, since the remaining organic matter is assimilated by the cells or used by them for the synthesis of biomolecules (OECD, 2006).

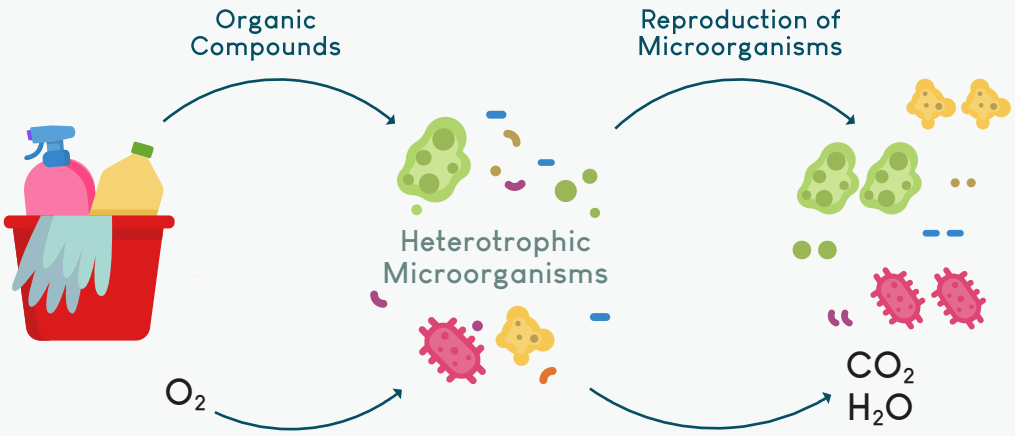

Figure 2. Transformation process of the organic compounds presents in cleaning products in *ultimate biodegradability tests.*

Figure 3 shows a graph with the results of *ready biodegradability* using the test ISO 10707:1994 to evaluate the biodegradability of organic chemical compounds present in cleaning products.

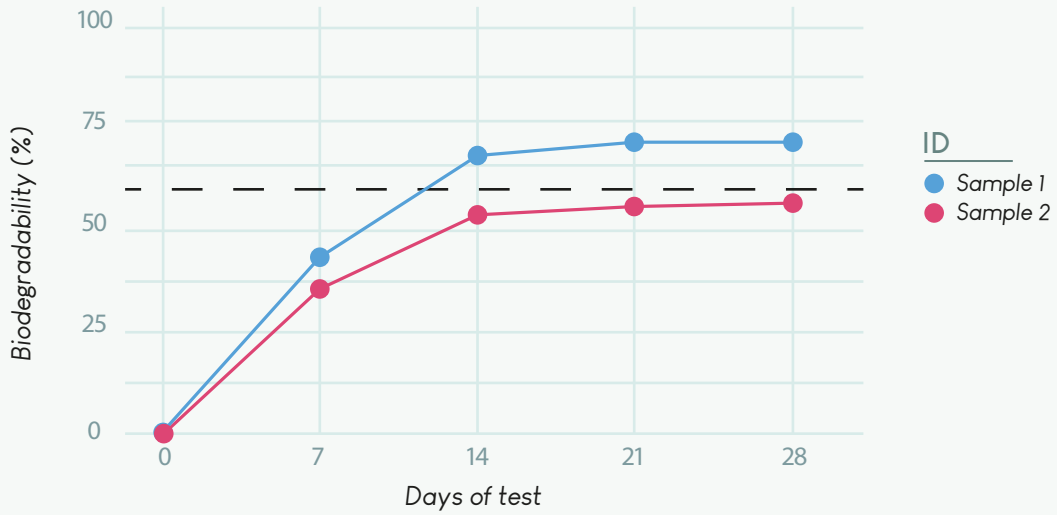

Figura 3. *Ready biodegradability* curves in organic chemical compounds present in cleaning products using the ISO 10707:1994 method. — — Black line corresponds to the 60 % value that allows an organic compound to be declared as "biodegradable" according to ISO 10707 (1994).

Laboratories that evaluate the biodegradability of organic chemicals require tools to declare compliance with the threshold level established by the methodologies, in order to take into account the level of risk associated with the decision rule used when reporting results (ISO/IEC 17025:2017). The uncertainty associated with the measurements is a fundamental factor in applying the decision rule (ISO/IEC Guide 98:2012) and therefore for declaring compliance with the established threshold level. In the case of biodegradability tests, it is an underdeveloped aspect, but its estimation provides a high level of confidence for the declaration of biodegradability of the organic chemicals tested.

It is important to bear in mind that when interpreting the results of ready biodegradability tests, the standardized conditions in which the tests are carried out at the laboratory level are far removed from the actual conditions to which the chemical substances will be exposed in the environment (Ahtiainen, Aalto, & Pessala, 2003; Brillet, Maul, Durand, & Thouand, 2016; Kowalczyk, et al, 2015). The compounds are tested at high concentrations, which would be unlikely to occur in the environment, and the standardized incubation conditions do not reflect the high variability of environmental

conditions, such as seasonality (Kowalczyk, et al, 2015). The biological degradation processes that take place in the different natural compartments are extremely complex phenomena, whereby different degradation kinetics can be observed when compared to those present in standardized tests (Brillet, Maul, Durand, & Thouand, 2016). Both the physicochemical conditions and the nature of the microbial populations typical of the environment are difficult to reproduce in the laboratory (Kowalczyk, et al, 2015; Pagga, 1997). In this regard, ready biodegradability tests constitute a relatively simple and conservative test, which is therefore suitable for the classification of chemical substances and their risk assessment (Kowalczyk, et al, 2015; Ahtiainen, Aalto, & Pessala, 2003; OECD, 2006). Despite their standardization, their wide use in regulatory frameworks and the large amount of information obtained since their introduction in the 1980s, attention is often drawn to the high levels of variability encountered (between replicates, between tests, between laboratories and over time) (Kowalczyk, et al, 2015; Comber & Holt, 2010). These statements are mainly based on the results obtained in a small number of interlaboratory test reports (Nyholm, Jørgensen, & Hansen, 1984; OECD, 1995).

The variability of the inoculum, both in terms of cell density as well as diversity, is often singled out as the main cause of the dispersion of the data in biodegradability tests (Mezzanotte, Bertani, Degli Innocenti, & Tosin, 2005; Thouand, Durand, Maul , Gancet, & Blok, 2011; Vázquez Rodríguez, Beltrán Hernández, Coronel Olivares, & Luc Rols, 2011; Brillet, Maul, Durand, & Thouand, 2016). The OECD guidelines allow for various sources of inoculum (activated sludge, sewage effiuent, surface water, soil, and mixtures) and a wide concentration range (see details in **Table 1)**. Variations in the source, concentration and pre-treatment of the inoculum have an impact on its diversity and consequently on its degradation capacity (Goohead, Head, Snape, & Davenport, 2014; Vázquez Rodríguez, Beltrán Hernández, Coronel Olivares, & Luc Rols, 2011). The possibility of increasing the comparability of the tests by standardizing the inoculum was addressed at the various meetings convened by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2003; ECETOC, 2007). However, such standardization cannot be carried out without at the same time reducing the number of species present in the tests, and it is therefore not recommended (OECD, 2006). The application of modern metagenomics techniques for analysing the degradation of surfactants in activated sludge reactors made it possible

to study the infiuence of the origin of the inoculum and the characteristics of the effiuent on the structure of the microbial community and the efficiency of the reactors (Chen, et al, 2019). Similarly, this type of analysis could be applied to ready biodegradability tests, with the aim of determining the infiuence of the initial diversity of microorganisms on the development of the community structure and its infiuence on the test results.

For the above reasons, the correct characterization of the precision and trueness of the methods, as well as the estimation of the measurement uncertainty, are fundamental for evaluating any recommendation to improve the methods. However, in biodegradability tests, the information available on the reproducibility of the test is too scant to be used for estimating measurement uncertainty. Therefore, this technical guide has been developed with the aim of helping laboratories to conduct ready biodegradability tests on water-soluble organic substances by providing simple technical guidelines for estimating uncertainty using the "top-down" approach to implementing the Biochemical Oxygen Demand method, or Closed Bottle Test (ISO 10707:1994).

5. Guide to estimating uncertainty using the "top-down" approach

Estimating measurement uncertainty is essential for evaluating the reliability of a measurement. There are various approaches to estimating measurement uncertainty, each with advantages and drawbacks (Priel, 2009). The *Guide to the Expression of Uncertainty in Measurement* (GUM, 2008) is the main guidance document that establishes the general rules for evaluating and expressing the uncertainty estimate. However, this method is geared towards estimating uncertainties in mathematical models in which all the sources of uncertainty are well known and can be combined individually to obtain a global combined uncertainty ("bottom-up" approach). In some quantitative measurements, the approach proposed by the GUM has been considered difficult to apply (Lee, et al, 2015) since it is not possible to individually identify, associate and evaluate all uncertainty contributions of a defined magnitude and later combine these contributions (EUROLAB, 2007). In order to broaden the scope of the GUM to estimate measurement uncertainty, alternative approaches have been designed that adhere to its principles. Specifically, one of the alternatives is the "top-down" approach, which is based on directly and globally estimating the measurement uncertainty based on the data that describe the performance of a method on the parameters of precision and trueness. These data are acquired through quality control studies, participation in proficiency testing, interlaboratory comparisons and method validation studies, among other means (EUROLAB, 2007). It is important to emphasize that these alternative approaches are accepted for determining compliance with the requirements of ISO/IEC 17025:2017 (Priel, 2009) and they are also a practical and simple way to estimate measurement uncertainty.

5.1. Procedure for estimating uncertainty using the "top-down" approach for the "ready" aerobic biodegradability method

To achieve a good estimate of uncertainty, it is necessary to have a clear definition of the measurand, as well as a comprehensive understanding of the test method procedure and a critical analysis of the possible variables that affect the measurement results. **Figure 4** shows the main steps to estimate measurement uncertainty with the "top-down" approach.

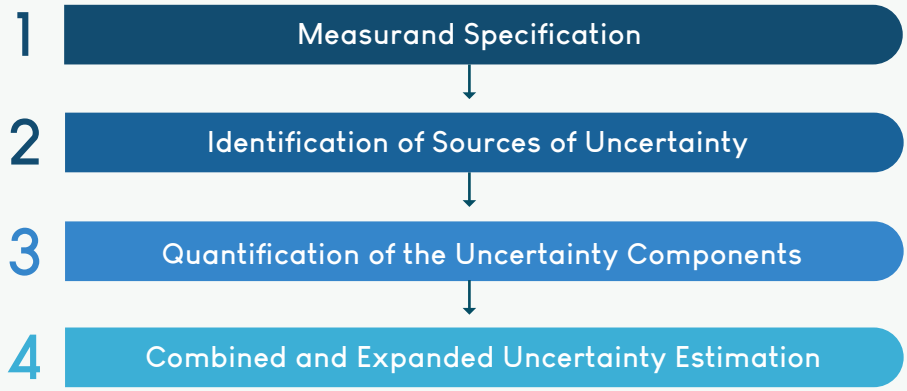

Figure 4. Sequential steps for estimating measurement uncertainty using the "top-down" approach.

The procedure for estimating measurement uncertainty using the "top-down" approach (Ellison & Williams, 2012) to measuring biodegradability using the Biochemical Oxygen Demand method, or Closed Bottle Test is detailed below (ISO 10707:1994). This was carried out using historical data for measurements using an activated sludge inoculum performed by the Department of Handling and Management of Chemicals (DMyGSQ) of the Argentine National Institute of Industrial Technology (INTI) and the results of the proficiency testing scheme organized in the framework of the regional project *"Quality assurance in the measurements required to determine the biodegradability of chemical"*. The samples analysed correspond to products for industrial and domestic use, mainly cleaning products containing organic substances.

Stage 1

Measurand Specification

Measurand specification consists of a clear and unambiguous description of what is to be measured. A practical way to clearly identify the measurand is to complete the following sentence: [measurand] in [matrix] using [method] (B. Magnusson, 2017). This definition intrinsically establishes an associated mathematical model, which allows the value attributable to the measurand to be estimated. This quantitative expression should make it possible to relate the value of the measurand to the parameters on which it depends (Ellison & Williams, 2012; EUROLAB, 2007).

For the purposes of "ready" aerobic biodegradability in organic substances, the mathematical model applied to the cleaning product matrix is described below. The scope includes raw materials as well as formulated products that do not show any toxicity in the test concentrations and that are soluble or form homogeneous and stable dispersions in an aqueous medium.

Definition of the measurand: Percentage of ultimate ready biodegradability of water soluble organic compounds using Biochemical Oxygen Demand (BOD), or Closed Bottle Test (ISO 10707:1994).

Eq. 1

$$D_{x_{t_i}}(\%) = \frac{(p_{x_{t_0}} - p_{x_{t_i}}) - (p_{b_{t_0}} - p_{b_{t_i}})}{DQO_x \cdot p_c} \cdot 100$$

where $D_{x_{t_i}}$ is the biodegradability of sample x being tested in the i^{th} time period in percentage units (%), $p_{x_{t_i}}$ is the dissolved oxygen concentration in the i^{th} time period in units of mg/L of the sample x being tested, $p_{x_{t_0}}$ is the dissolved oxygen concentration at time zero in units of mg/L of the organic substance x being tested, $p_{b_{t_0}}$ is the dissolved oxygen concentration at time zero in units of mg/L of the blank, $p_{b_{t_i}}$ is the dissolved oxygen concentration in the i^{th} time period in units of mg/L of the blank, DQO_x is the chemical oxygen demand for the sample x in units of mg/mg, p_c is the concentration of sample x in units of mg/L in the test bottle.

Stage 2

Identification of sources of uncertainty

To achieve proper identification of the main sources of uncertainty, a careful analysis of the measurement procedure must be carried out (Ellison & Williams, 2012). The sources of uncertainty associated with the measurand are usually represented using a "cause and effect" diagram (EUROLAB, 2007). With regard to the method under study, the estimation of uncertainty using the "top-down" approach is limited to the sources of uncertainty associated with the precision and bias of the method, and its respective components as shown in the "cause and effect" diagram in **Figure 5** (EUROLAB, 2007).

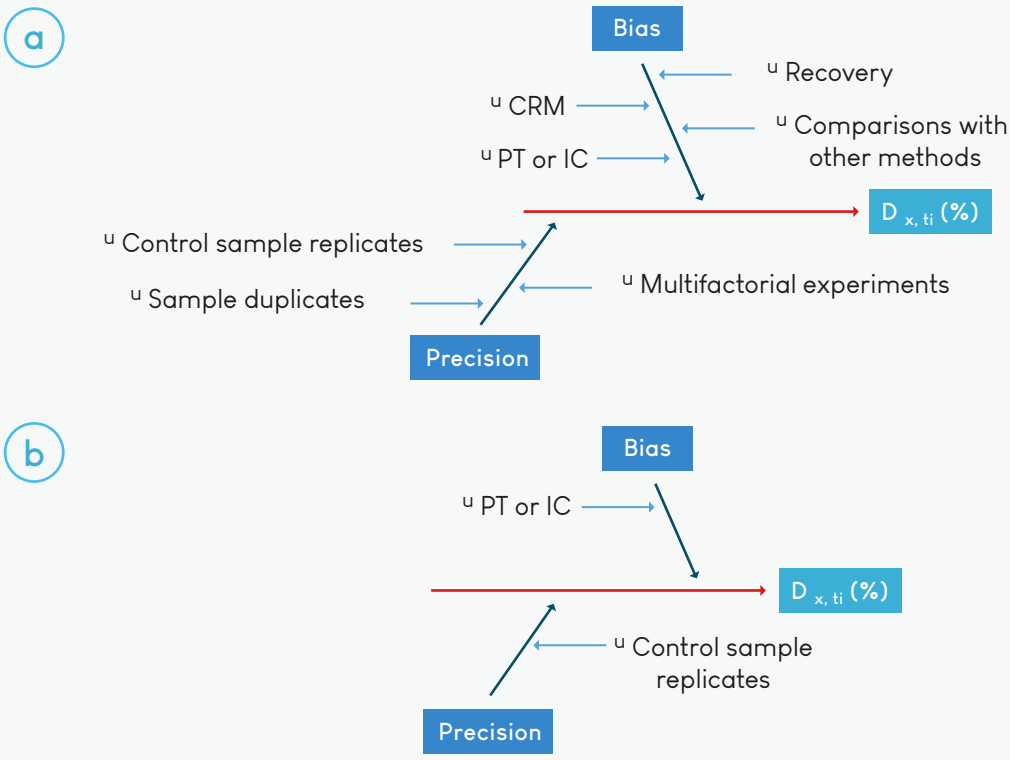

Figure 5. "Cause and effect" diagram according to the "top-down" uncertainty estimation approach; **a** – Diagram showing all the possible strategies for estimating the uncertainty components associated with precision and bias; **b** – Diagram showing the relevant uncertainty components for the case under study.

These two sources of uncertainty are considered dominant contributions when estimating the uncertainty and, furthermore, these are parameters that describe the general performance of the method, which is why their modelling and effects on the measurement result can be better interpreted separately (Ellison & Williams, 2012). It is important to note that **Fig. 5-a** shows all possible strategies for quantifying these uncertainty components, but only those that allow us to estimate the uncertainty components relating to the information available for the case in question are later used (**Fig. 5-b**).

Stage 3

Quantification of the uncertainty components

The information available on the method performance must be used for two things:

- Based on the analysis conducted in stage 2, an association is made between the identified sources and the available data, which would make it possible to identify those components whose uncertainty contribution can be quantified (Ellison & Barwick, 1998).

- For those sources whose uncertainty contribution cannot be estimated using the available information, a plan to obtain the necessary data should be drawn up. This may involve developing experimental designs or searching for information in the literature, certificates, technical specifications of equipment, and so on (Ellison & Williams, 2012).

Figure 6 shows the main sources of uncertainty that can be quantified based on the historical data for the measurement of biodegradability in water soluble organic compounds using the ISO 10707 method with an activated sludge inoculum from INTI's DMyGSQ and the results of the proficiency testing scheme organized within the framework of the regional project.

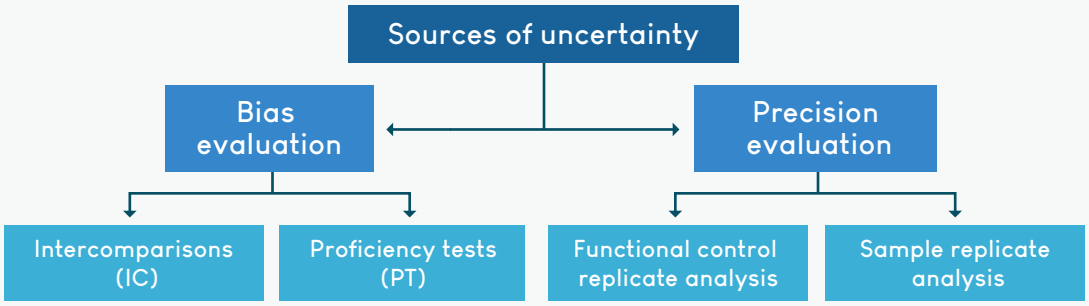

Figure 6. Uncertainty components determined for measuring "ready" biodegradability in water soluble organic substances for the case study.

Below is an explanation of how to estimate the uncertainty contribution based on the source of uncertainty and the method performance information available:

- **Precision of the method in a laboratory**

This uncertainty component expresses the intermediate precision and repeatability of the method in the laboratory using historical data on a sample whose behaviour or property to be measured has been defined (for example, control samples). To estimate this uncertainty component, the following data can also be used:

- **Control samples that cover the whole scope of the test method:** these correspond to measurements made at specific times to study the variability of the measurement system. For the method under study, covering the scope of work would mean having both biodegradable and non-biodegradable control samples.

- **Measurements of control samples and routine samples:** these correspond to measurements (repetitions) made on a biodegradable control sample and data from routine samples (at least in duplicate) with different levels of biodegradability.

The historical data from INTI's DMyGSQ made available for the study to estimate the precision uncertainty component of the method are described in **Table 2**. For the purposes of the example that is presented in this guide, the component of uncertainty due to the precision source was defined, in

the first instance, based on the analysis of the historical data on functional controls (control sample) and routine samples, since these data were obtained under conditions of repeatability and intermediate precision as recommended by Ellison & Williams (2012). It is important to highlight that according to the test method ISO 10707:1994, a functional control is a known organic substance with a simple chemical structure, which must exceed 60% biodegradability after 14 days of the test.

Table 2. Description of the historical data from INTI's DMyGSQ made available for the study to estimate the uncertainty component due to precision of the "ready" biodegradability measurement method in water-soluble organic substances.

Data source	Organic reference substance	Data Count	Replicates for each data point	Test time	Data date
Functional controls (control samples)	Sodium acetate ($NaC_2H_3O_2$)	28	Triplicates and duplicates	28 days	2013 to 2018
Routine samples	N/A	43	Triplicates and duplicates	28 days	2013 to 2019

The historical functional control data from INTI's DGyMSQ correspond to the analysis of sodium acetate, whose observed variability reflects the repeatability and intermediate precision of the method over a period of time. This variability takes into account the differences in several laboratory conditions such as: analysts, equipment used, water source, inoculum (origin, temperature, feeding, biological composition, trophic and metabolic state), among other factors.

It is important to highlight that the data used underwent analysis to demonstrate their validity for use in estimating measurement uncertainty as recommended by Ellison & Williams (2012). Therefore, to understand the distribution of and possible trends in the data from the measurement of functional controls, the average biodegradability values were then plotted according to their test date, as shown in **Figure 7**.

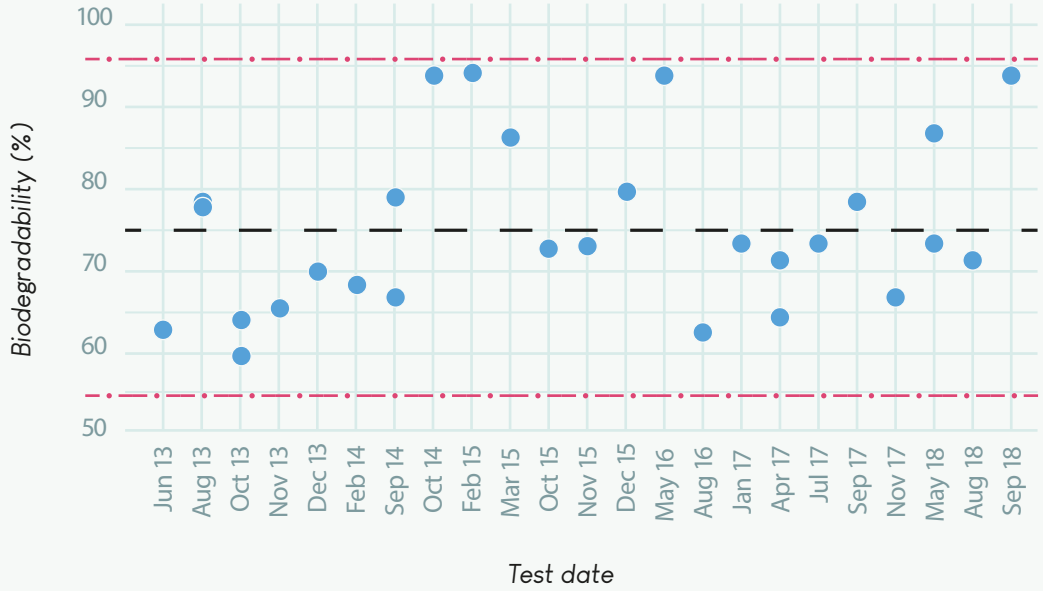

Figure 7. Control chart showing average biodegradability values obtained using the ISO 10707 method for functional controls according to test date. — — Black line corresponds to the average value of the historical data; -·-·- Dotted red lines correspond to the dispersion intervals calculated as twice their standard deviation (2·s). Data source: INTI's DMyGSQ.

To estimate the repeatability and intermediate precision components of the method using INTI's functional controls biodegradability data (**Table A1** in **Annex 1**) with an activated sludge inoculum, a one-way analysis of variance (ANOVA) was performed, as described by Ellison & Williams (2012), ISO 5725-2:2006 and Magnusson & Örnemark (2014). Based on the results of the one-way ANOVA (see **Table A2** del **Annex 1**), the uncertainty component associated with the precision source of the method was using Eq. 2. This equation, follows the guidelines for the linear combination of uncertainty contributions GUM (2008), where s_r^2 is the variance due to repeatability and s_L^2 is the intermediate precision variance associated under consideration (data number or test date). **Table 3** presents a summary of all the estimated values used to calculate the precision uncertainty component of the method.

Table 3. Summary of the precision uncertainty calculation of the ISO 10707 biodegradability test method for organic substances present in cleaning products.

Data source	Values according to variable (%)		Precision uncertainty (%)
	s_r^2	s_L^2	u_{prec}
Functional control	4.85	9.05	10.27

Eq. 2

$$u_{prec} = \sqrt{s_r^2 + s_L^2}$$

Finally, using the routine samples replicate data (see **Annex 2**), a repeatability component was estimated in order to identify whether the source of precision uncertainty of the ISO 10707 method is different when analysing samples that are not readily biodegradable (biodegradability of less than 60%) or samples with a more complex chemical composition than that of sodium acetate, such as mixtures of organic chemicals, household or industrial chemicals with complex formulations. Comparative analysis of the degree of infiuence on the precision uncertainty component is detailed in **Annex 2** of this document. The conclusion reached is that the variability observed in the routine samples was lower than in the functional controls, which is why the variability due to quantified repeatability in the routine samples is considered to already be covered within the precision uncertainty component u_{prec} estimated in **Table 3**.

- **Method bias estimated based on proficiency testing (PT) or interlaboratory comparisons (IC)**

Data from PT or IC can be used as a source to estimate the uncertainty component due to method bias (see **Fig. 5**). The data obtained from PT or IC exercises are recommended as a way to estimate the uncertainty contribution (EUROLAB, 2007; ISO/IEC 17043:2010; ISO 13528:2015; Magnusson & Örnemark, 2014) when:

- The test method used is the same or they are metrologically comparable methods.
- The assigned values have an appropriate measurement uncertainty.
- There have been at least six (satisfactory) participations in PT or IC exercises over a reasonable period of time.
- The use of consensus values is recommended when based on a large enough quantity (18 ≤ participating laboratories), as recommended by ISO 13528:2015.
- The provider of the PT or IC has proven competence in the ISO/IEC 17043:2010 requirements.

Sometimes the range of proficiency testing or interlaboratory comparisons available for certain physicochemical-biological tests is not very wide, thus limiting the possibility of estimating this uncertainty component. For the purposes of this guide, in order to provide a practical and realistic example within the scope of the ISO 10707 method, the estimation of the bias uncertainty component of the "ready" biodegradability method was estimated using the results of the proficiency test scheme DMQ-001-2019 (LACOMET, 2019), as it is the only interlaboratory comparison exercise organized in the region in recent years that includes the application of the test method ISO 10707:1994 within its scope. In addition, the PT scheme shows assigned values with a measurement uncertainty lower than the maximum variability (20%) defined by the test method ISO 10707:1994. It should be noted that this proficiency test was performed with two test items, one simulating a cleaning product formulation (BioPL) and the other prepared using a commercial technical grade surfactant (BioTA). Also made available were the results of several laboratories participating

in the regional project *"Quality assurance in the measurements required to determine the biodegradability of chemicals"* including INTI's DMyGSQ. It should be clarified that the assigned values ($x_{ref\ PT}$) of the proficiency test items were designated based the measurements made by an expert laboratory (INTI's DMyGSQ). Also in order to achieve comparability between the results of the participants ($x_{lab\ i}$) and the values assigned to the PT items, the PT organizer requested that only the commercial inoculum capsule provided to each participant (same lot and same firm) be used, as detailed in LACOMET (2019).

Based on the information from PT DMQ-001-2019, the estimation of the uncertainty component was calculated using Eq. 3 and Eq. 4 (B. Magnusson, 2017; LACOMET, 2019).

Eq. 3

$$RMS = \frac{\sum bias_i^2}{p} = \frac{\sum (x_{lab\ i} - x_{ref\ PT})^2}{p}$$

Eq. 4

$$u_{bias} = \sqrt{RMS}$$

Table 4 shows the detailed results of the calculations needed to estimate the bias uncertainty component of the ISO 10707:1994 test method, using the satisfactory results obtained by the participants in the proficiency testing scheme DMQ-001-2019, Eq. 3 and Eq. 4. **Table A3** in **Annex 1** provides more information about the data used to estimate the bias uncertainty component of the method, all resulting from satisfactory performance evaluations obtained within the proficiency testing scheme framework.

Table 4. Summary of the calculation of the global bias uncertainty of the ISO 10707 biodegradability test method using the satisfactory results obtained by the participants in the proficiency testing scheme DMQ-001-2019 for organic substances in cleaning products (BioPL) and a technical grade commercial surfactant (BioTA).

Proficiency test item	Bias (%)	RMS (%)	u_{bias} (%)
BioPL	-6.4	45.6	6.75
BioPL	9.2		
BioPL	5.7		
BioPL	8.9		
BioTA	-3.4		
BioTA	5.0		

Stage 4

Combined and Expanded Uncertainty Estimation

At this stage, the standard uncertainties for each of the sources of uncertainty are combined. The recommendation is to follow the GUM (2008) guidelines, if applicable, to estimate the combined uncertainty. Since it is reasonable to assume that the uncertainty components identified in **Figure 5** are independent, the uncertainty combination is estimated as shown in Eq. 5.

Eq. 5

$$u_c = \sqrt{(u_{bias})^2 + (u_{prec})^2}$$

To estimate the expanded uncertainty, it is advisable, if applicable, to follow the provisions of the GUM method. For the sake of practicality, in most cases the recommendation is to follow the central limit theorem and estimate the expanded uncertainty according to Eq. 6 with a $k = 2$ to give a confidence of approximately 95% (GUM, 2008).

Eq. 6

$$U = k \bullet u_c$$

Table 5 shows the combined uncertainty and expanded uncertainty for the ISO 10707 biodegradability test method for water soluble organic compounds, using the precision and bias uncertainty components estimated in step 3 of this procedure for estimating uncertainty with the "top-down" approach. The expanded uncertainty is expressed with two significant figures, following the GUM recommendation GUM (2008).

Table 5. Combined uncertainty and expanded uncertainty using the "top-down" approach to the ISO 10707 biodegradability test method for organic substances in cleaning products.

Uncertainty components		u_c (%)	U (%)
u_{prec}	10.27%	12.29	25
u_{bias}	6.75%		

6. Conclusions

This guide presents a practical and reliable alternative for estimating measurement uncertainty using the "top-down" approach to the ISO 10707 test method and applicable OECD guidelines when determining the "ready" aerobic biodegradability of water soluble organic compounds present in cleaning or similar products.

The possibility of estimating the uncertainty of the test, following the strategy and information proposed in this guide, enables different analysis laboratories to determine the level of confidence of their results and incorporate this information into the decision rule for the declaration of biodegradability of the samples evaluated.

The estimation of measurement uncertainty using the "top-down" approach is a method that allows us to ascertain and evaluate the performance of the method on the parameters of bias and precision; it also allows us to make use of any historical data that laboratories may have based on measurements carried out on real test samples, controls to ensure the validity of the results and participations in PT or IC.

The expanded uncertainty estimated in this guide using the data from INTI's DMyGSQ and the results of the proficiency testing scheme DMQ-001-2019 is greater than but very close to the maximum difference of 20 % allowed between replicates, as set out in the ISO 10707:1994 test method. However, it must be emphasized that the expanded uncertainty estimated using the "top-down" approach includes the bias and precision uncertainty components of the method, while the maximum difference allowed is only associated with the precision of the method under repeatability conditions.

The bias uncertainty component was estimated based on a proficiency testing that used a commercial inoculum, but it is important to bear in mind that the reference methodologies state that inocula of environmental origin must be used, and it is thus necessary to ascertain and incorporate their

impact on that uncertainty component by increasing the participation of methodologies that use inocula of environmental origin in interlaboratory comparisons or proficiency testing in order to better estimate the bias uncertainty component.

Furthermore, to achieve a greater understanding of and insight into the ISO 10707:1994 biodegradability test method, it is necessary to carry out scientific research that focuses on determining the degree of variability associated with the microbial diversity of the inocula that are routinely used in the test and its effect on measurement. This information would help to develop new strategies to improve the performance of the method on the parameters of precision and bias.

Finally, the strategy for estimating measurement uncertainty presented in this guide may be used to update the classifications of chemicals that are widely used for both industrial and domestic purposes, by providing reliable environmental information that promotes their responsible use.

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9. Annex 1

Table A1. Biodegradability data at 28 days of testing with the ISO 10707 method on functional controls, obtained from the data provided by INTI's DMyGSQ.

Data	Biodegradability values (%) according to the measurement replicate			Average biodegradability
	1	2	3	
1	62.71	61.00	62.57	62.09
2	78.29	71.29	70.57	73.38
3	77.86	77.86	--	77.86
4	59.43	60.57	62.29	60.76
5	64.00	71.86	63.71	66.52
6	65.43	65.57	66.57	65.86
7	70.29	75.86	57.14	67.76
8	68.70	69.58	--	69.14
9	79.20	67.11	73.30	73.20
10	66.67	67.58	78.08	70.78
11	93.80	99.43	96.86	96.70
12	94.04	96.30	88.46	92.93
13	86.16	81.20	89.06	85.47
14	72.97	85.27	87.98	82.07
15	73.21	81.83	63.01	72.68
16	79.52	72.15	84.94	78.87
17	94.24	95.96	--	95.10
18	62.35	69.66	69.52	67.18
19	73.71	72.29	70.14	72.05
20	70.95	70.67	72.22	71.28
21	64.26	68.21	55.34	62.60
22	73.55	75.48	79.49	76.17
23	78.72	83.95	78.43	80.37
24	67.50	67.08	71.90	68.83

Data	Biodegradability values (%) according to the measurement replicate			Average biodegradability
	1	2	3	
25	73.94	75.80	--	74.87
26	86.83	84.67	80.35	83.95
27	71.08	72.23	--	71.66
28	93.99	90.37	--	92.18

Cells with no values (--) represent measurement replicates that failed to comply with the controls to ensure the validity of the results applied by INTI's DMyGSQ.

Table A2. Summary of the one-way ANOVA results of the functional control data used to estimate the precision uncertainty component of the ISO 10707 method.

Origin of variations	Sum of squares	Degrees of freedom	Mean square
Between groups	7271.396937	27	269.3109977
Within groups	1175.684367	50	23.51368733
Total	8447.081304	77	

Table A3. Data from PT DMQ-001-2019 used to estimate the bias uncertainty component of the ISO 10707 method.

Participant code	Proficiency test item	Reported value (%)	Assigned value (%)	Bias (%)
001	BioPL	62.50	68.9	-6.4
003	BioPL	78.10		9.2
004	BioPL	74.60		5.7
005	BioPL	77.78		8.9
002	BioTA	44.60	48	-3.4
003	BioTA	53.00		5.0

BioPL: Cleaning product, BioTA: Technical grade commercial surfactant.

10. Annex 2

Table A4 shows the biodegradability results for routine samples that were used to analyse whether the chemical composition and degree of biodegradability of the samples affect the uncertainty component due to the repeatability of the precision source. For this purpose, a standard deviation was estimated for each data set using Eq. A1, since these data can be considered to have been obtained under repeatability conditions.

Eq.A1

$$s_{M,i} = \sqrt{\frac{\sum\left(x_{ij} - \bar{x}_i\right)^2}{n_i - 1}}$$

Using Eq. A2, an average variance was estimated due to repeatability for aggregate data with different sample sizes as recommended by Montgomery (2004). This variance describes the method results differences associated with determinations of the biodegradability of samples with complex chemical compositions and with biodegradability percentages of between (1.5 and 89.4)%.

Eq.A2

$$\bar{s}_M = \sqrt{\left[\frac{\sum_{i=1}^{m}(n_i - 1) \bullet {s_{M,i}}^2}{\sum_{i=1}^{m} n_i - m}\right]}$$

Table A5 shows the values for variance due to repeatability and the values for precision uncertainty of the method estimated based on each data set. For the case studied in this guide, under repeatability conditions the method could be interpreted as having greater variability when sodium acetate is analysed as a functional control($s_r > \bar{s}_M$). This demonstrates that the matrix effect of the samples due to the chemical composition and degree of biodegradability does not infiuence the estimation of the variance due to repeatability of the method. Taking a very practical approach,

it could be concluded that the precision uncertainty component of the method estimated on the basis of the functional control data covers the variability of the method when it is used to determine the biodegradability of samples that are not readily biodegradable (biodegradability of lower than 60%) or samples with a more complex chemical composition than sodium acetate, such as mixtures of organic chemicals, and household or industrial chemicals with complex formulations.

Table A4. Biodegradability data at 28 days of testing with the ISO 10707 method on samples obtained from the data provided by INTI's DMyGSQ.

Data	Biodegradability values (%) according to the measurement replicate			s_M	$n_i - 1$	$(n_i - 1) \cdot s_M^2$
	1	2	3			
1	65.36	62.21	60.78	2.34	2	10.98
2	83.53	69.25	--	10.10	1	101.96
3	89.41	84.27	--	3.63	1	13.21
4	80.97	70.17	72.69	5.65	2	63.85
5	70.15	64.31	--	4.13	1	17.05
6	85.50	77.38	80.35	4.11	2	33.76
7	72.68	72.60	76.06	1.97	2	7.80
8	79.05	78.45	79.05	0.35	2	0.24
9	54.16	47.81	55.52	4.12	2	33.87
10	49.92	55.19	56.68	3.55	2	25.23
11	61.43	61.95	60.28	0.85	2	1.46
12	49.81	49.30	52.90	1.95	2	7.59
13	74.21	81.31	78.68	3.59	2	25.77
14	56.86	46.86	52.29	5.01	2	50.12
15	69.40	65.57	--	2.71	1	7.33
16	71.60	72.70	64.99	4.17	2	34.78
17	67.86	68.29	64.71	1.95	2	7.64
18	75.72	71.66	69.25	3.27	2	21.38
19	63.48	54.37	58.02	4.58	2	42.04
20	62.53	71.84	73.61	5.95	2	70.86
21	63.28	60.78	--	1.77	1	3.13
22	70.54	69.81	75.62	3.16	2	20.03

Data	Biodegradability values (%) according to the measurement replicate			s_M	$n_i - 1$	$(n_i - 1) \cdot s_M^2$
	1	2	3			
23	51.79	57.08	59.94	4.13	2	34.20
24	18.43	22.43	22.14	2.23	2	9.95
25	64.72	57.67	60.92	3.53	2	24.90
26	55.33	53.34	--	1.41	1	1.98
27	44.16	44.59	45.30	0.58	2	0.66
28	25.96	27.53	25.53	1.05	2	2.22
29	33.00	33.57	33.29	0.29	2	0.16
30	61.57	67.43	64.71	2.93	2	17.20
31	57.00	58.57	55.57	1.50	2	4.50
32	64.71	71.43	--	4.75	1	22.58
33	49.79	52.65	--	2.02	1	4.09
34	57.29	54.43	62.00	3.82	2	29.22
35	56.86	55.43	50.86	3.13	2	19.64
36	39.20	44.64	--	3.85	1	14.80
37	54.21	56.35	55.06	1.08	2	2.32
38	82.30	77.73	80.97	2.35	2	11.05
39	70.57	74.86	79.57	4.50	2	40.53
40	22.43	23.86	--	1.01	1	1.02
41	1.57	6.14	3.57	2.29	2	10.50
42	26.68	17.40	26.96	5.44	2	59.20
43	62.46	68.77	66.48	3.19	2	20.41
					$\sum_{i=1}^{m}(n_i - 1)\cdot s_{M,i}^2$	931.22
					m	43
					$\sum_{i=1}^{m} n_i$	119
					$\sum_{i=1}^{m} n_i - m$	76
					$\bar{s}_M$	3.50

Cells with no values (--) represent measurement replicates that failed to comply with the controls to ensure the validity of the results applied by INTI's DMyGSQ.

Table A5. Summary of variances and uncertainty of the precision component of the ISO 10707 method according to the source of the data analysed.

Data source	Variances (%)		Precision uncertainty (%)
Functional controls	s_r	4.85	10.27
	s_L	9.05	
Routine samples replicates	$\bar{s}_M$	3.50	9.71
	s_L	9.05